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Orion II Microplate Luminometer Simplicity 4

ToxiLight™ Non-Destructive Cytotoxicity Bio Assay Kit (Lonza)

ATP detection systems are commonly used for detecting cytotoxicity. These measure the ATP from viable cells (or lack of them) rather than a direct measurement of cytotoxicity, and in some cases cell death may not be the correct assumption to make when ATP levels decrease.

An alternative indicator of cytotoxicity is damage to the plasma membrane. This allows reagents to enter the cells (e.g. propidium iodide), and allows leakage of cell components into the surrounding medium. One of the enzymes released when cell membrane damage occurs is adenylate kinase (AK), a ubiquitous protein present in all eukaryotic cells.

The ToxiLight™ Assay is based on the bioluminescent measurement of adenylate kinase. The measurement of the release of AK from cells allows the accurate and sensitive determination of cytotoxicity and cytolysis.

The reaction involves two steps. The first involves the addition of ADP as a substrate for AK. In the presence of the enzyme, AK, the ADP is converted to ATP for assay by bioluminescence:

Reaction 1

$$Mg^{++}ADP + ADP \xleftrightarrow{\text{Adenylate Kinase}} Mg^{++}ATP + AMP$$

The bioluminescent method utilizes an enzyme luciferase, which catalyses the formation of light from ATP and luciferin according to the following reaction:

Reaction 2

$$ATP + Luciferin + O_2 \xrightarrow[Mg\ ++]{\text{Luciferase}} Oxyluciferin + AMP + PP_i + CO_2 + Light$$

By combining the two reactions, the emitted light intensity is linearly related to the AK concentration and is measured using a luminometer

The ToxiLight™ Assay is a homogeneous, non-destructive assay, enabling the direct assay of wells containing viable and non-viable mixed populations. The assay can also be used on samples of culture medium taken from experimental wells, enabling the remaining cultures to be assayed using additional methods. This is a useful feature when samples are precious and multiple analyses are preferred.

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Materials

Luminometer: Orion II Microplate Luminometer
Software: Simplicity 4
Assay : ToxiLight™ Non-Destructive Cytotoxicity Bio Assay Kit (Lonza)
Microplates: opaque microplates (solid, white, 96 well), supplied by Porvair

Method

Either:
100µl of culture (exposed to drug for a determined time period) was taken as the sample into triplicate wells.

Or:
20µl of supernatant from a culture (exposed to drug for a determined time period) was taken as the sample into triplicate wells.
100 µl of AK Detection Reagent (reconstituted in Tris-Ac Buffer) was added to each well and incubated at room temperature for 5 minutes.
The plate was placed into the Orion II and a 1s integrated read taken.

For detailed assay instructions please visit:

http://www.lonzabioscience.com/Content/Documents/Bioscience/18947-0107-01.pdf

Example

The assay as detailed above was conducted using triplicate dilutions of K562 cells (lysed with ToxiLight™ 100% lysis reagent to release optimal AK) at predetermined cell numbers. Samples were read after 5minutes and after 15minutes.

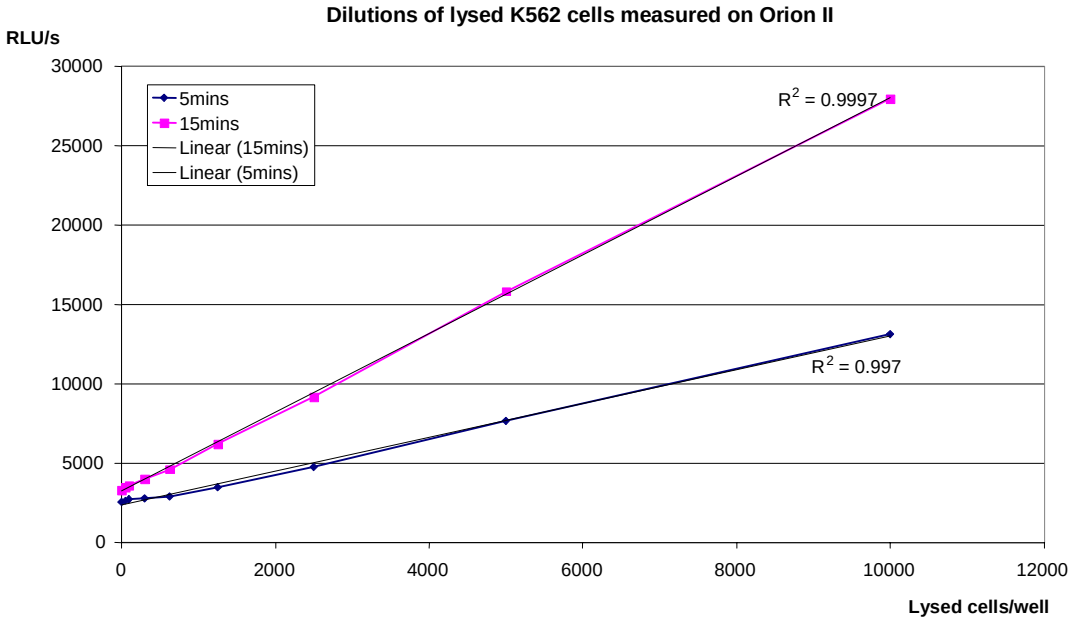

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Figure 1: Investigating the sensitivity of the Orion II for use with Lonza ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit.

Result

The Orion II microplate luminometer showed excellent sensitivity and linearity in the detection of a wide range of cell numbers using Lonza ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit.

Acknowledgement
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